Effect of Genetics and Maternal Dietary Iodide Supplementation on Turkey Embryonic Growth[1]

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ABSTRACT Embryonic growth of a turkey lines selected for 16-wk BW (F) or 180-d egg production (E) was measured and compared to randombred controls (RBC2 or RBC1). Egg weight at setting relative to poult weight at hatching indicated increased growth in F as well as E embryos compared to randombred controls. Eggs from F weighed 10 g more than those of RBC2 ($P \leq 0.0001$) but the poults at hatching were only 8 g heavier ($P \leq 0.0001$). Water vapor loss during incubation indicated that only 0.9% of the difference could be accounted for by water vapor loss. Selection for increased 16-wk BW resulted in decreased embryo growth rates relative to hatchling mass ($P \leq 0.0001$) beginning at Day 16 of incubation compared to that of RBC2. Eggs from E weighed 15 g less than RBC1 ($P \leq 0.0001$) but produced poults weighing only 7 g less ($P \leq 0.0001$). Incubation water vapor loss was depressed in E compared to RBC1 ($P \leq 0.0001$) but accounted for only 1.4% of the difference between hatchling weights. Selection for egg production increased embryo growth rates (relative to hatchling mass) measured at 4-d intervals compared to those of the RBC1 line ($P \leq 0.05$). Iodide supplementation of the maternal diet depressed ($P \leq 0.05$) glycogen in F embryos but interacted with line to generally increase glycogen in E embryos. Increased glycogen was related to increased growth rates in E but not F line embryos. It may be concluded that iodide supplementation of the material diet and genetics are determinants of embryonic growth in turkeys.

(*Key words*: growth, embryo, genetic lines, thyroid)

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INTRODUCTION

Genetic selection for growth is negatively correlated with embryonic survival of turkeys (Nestor and Noble, 1995). Hatchability of lines selected for increased growth rate has declined, whereas that of lines selected for egg production has improved. Understanding the constraints on embryonic growth in such selected lines may help improve the survival rates of turkey embryos.

Currently, nearly one-fifth of the life cycle of turkeys is spent *in ovo*, yet little is known about the effects of genetic selection for growth or egg production on the growth patterns of turkey embryos. Ricklefs (1987) described mathematically derived growth curves fitted to compare embryonic growth among different avian species, including the turkey. He concluded that embryonic growth across avian species was similar and that the greatest contributors to its variation were egg weight and length of the incubation period. The selection of turkeys has resulted in dramatic changes in egg weights (Nestor and Noble, 1995) and incubation periods (Christensen *et al*., 1994) but little is known about the effects on growth rates of embryos.

When turkey hens selected for increased growth rate were fed triiodothyronine- or thiouracil-supplemented diets (Christensen *et al*., 1991), embryo growth and glycogen metabolism was influenced. Reduced embryonic growth rates may be caused by decreased availability of sufficient nutrients to support metabolism and growth or limiting organ maturation (Ricklefs, 1987).

The purposes of the experiments reported here were: 1) to determine differences in embryonic growth of turkey embryos from lines selected for growth or egg

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Abbreviation Key: E = turkey line selected for 180-d egg production; F = turkey line selected for 16-wk BW; RBC = randombred control line;

production compared to their randombred control populations and 2) to observe the added influences of supplementation of iodide to the maternal diet on the growth and energy metabolism (as influenced by whole body glycogen content) of the different strains.

MATERIALS AND METHODS

The breeder turkeys used in these experiments were from 3 yr representing the 32nd, 33rd, and 34th generations of a line selected for increased 16-wk BW (F line) or 35th, 36th, and 37th generations of a line selected for increased 180-d egg production (E line) and their respective randombred control lines (RBC2 and RBC1) (Nestor, 1984; Nestor and Noble, 1995). Hatching eggs were obtained in May of each year, the poults were hatched in June, and then poults were grown using commercially accepted practices (Grimes *et al*., 1989; Christensen *et al*., 1993). At 30 wk of age, egg production was induced by photostimulation (15.5 h of light/d, 0500 to 2030 h). Thereafter, hens within lines were divided randomly into 24 pens. Thyroid hormone concentration in plasma from laying hens and embryos was measured according to Christensen *et al*., 1992). Hens were artificially inseminated with semen from hatchmate toms of the respective lines. Eggs were incubated in Jamesway 252B incubators.

Experiment 1

At biweekly intervals for a 20-wk laying period, 18 randomly selected eggs per genetic line were weighed (nearest 0.1 g) immediately prior to setting and at transfer to the hatcher (24 d of incubation) and the poults from the same eggs were weighed at hatching. A total of approximately 300 eggs and poults from each line were weighed. To monitor weight loss during inculation due to water, eggshell conductance was measured and conductance constants of the same eggs were computed by the calibrated egg technique of Tullett (1981). The poult to egg weight ratios were computed for eggs that hatched to evaluate the weight of the poult at hatching relative to the initial egg mass at the beginning of incubation. The relative weight was calculated by dividing the initial egg mass (grams) into the poult weight at the end of 28 d of incubation and multiplying by 100.

Data were analyzed using the General Linear Models procedure of SAS® (SAS Institute, 1989) using genetic line as the single factor in the experiment. Means were separated using the least squares mean procedure of SAS®.

Experiment 2

In contrast to the observations of Ricklefs (1987), differences were observed in Experiment 1 in the size of the hatchlings independent of egg weight and water loss. The objective of Experiment 2 was to determine at what stage of embryonic development the growth differences seen in Experiment 1 were occurring. It was hypothesized that these differences may have been because of thyroid-related growth differences. Breeder hens within each of the four lines were randomized and placed into each of 24 pens as described in Experiment 1. Half of the pens received the same basal diet described in Experiment 1 containing 0.4 ppm iodide (the recommended NRC requirement) and the remaining half received the identical basal diet containing 10-fold the NRC requirement of supplemental iodide (4 ppm) supplemented as potassium iodide. The 4 ppm has been shown to elevate thyroid hormone concentrations and be a nontoxic dose for turkey breeder hens (Christensen and Ort, 1991). Iodide effects on the hen plasma hormones were verified by RIA on blood collected monthly from hens. Eggs were examined for an 18-wk laying period using 320 eggs from each line by iodide treatment combination. Eggs from all treatment combinations were set at biweekly intervals (nine hatches) and incubated as described previously. From Hatches 1, 5, and 9, embryos from each line were selected at random and weighed at 4-d intervals after removal from the egg beginning at Day 8 of incubation. Embryos were lifted from the egg with a spatula and the yolk sac was trimmed from the body at the yolk stalk. Because of the fragility of early embryos (Days 8 through 16), trimming was done in a warm sterile saline solution using a fine brush and surgical scissors. Following trimming, each embryo was blotted lightly with a wet paper towel, weighed (nearest 0.1 mg), and frozen (–20 C). Because of the reported involvement of embryonic growth with thyroid function, it was of interest to determine total body glycogen.

Thawed carcasses were assayed as follows for total body glycogen content following storage: the whole embryo was dispersed in a tissue homogenizer (ULTRA TURRAX T25)[3] in one (Day 8, 12, and 16 embryos), two (Day 20 embryos), and six (Day 24 and 28 embryos) volumes of distilled water containing 7% perchloric acid. The homogenates were recovered following centrifugation at $700 \times g$ for 10 min at 4 C. Duplicate aliquots of 50 μL of each homogenate were assayed for glycogen by the method of Dreiling *et al*. (1987). The total amount of glycogen measured in the carcass was divided by the BW to determine the amount of glycogen analyzed per gram of BW at each of the developmental days observed.

Because of the large differences seen in egg weights among the lines, growth rates and rate of glycogen accrual and disappearance were determined following the method of Ricklefs (1987) described above for BW. Growth and glycogen were expressed at each sampling time as a percentage based on the average weight of each treatment combination at hatching. Observed BW were divided by the mean hatchling BW (n = 10) and multiplied by 100 to determine the percentage of growth that had occurred at each time interval. The percentage decline in glycogen concentration was calculated similarly.

[3]IKA-Works, Inc., Cincinnati, OH 45240.

Data were analyzed as two levels of lines of turkeys (F vs RBC2 or E vs RBC1) by two levels of iodide (0 and 4 ppm supplementation) in a completely randomized factorial arrangement of treatments using the SAS/STAT® program (SAS Institute, 1989). Trial was a fixed factor in the experiment but no significant interactions due to trial were noted, so the data were pooled for final analysis. Means differing significantly were separated the least square mean procedure. Significance was based on $P \leq 0.05$. Percentages were transformed to arc sine square root percentages for analysis.

RESULTS

Experiment 1

F Line vs RBC2 Embryo BW. F Line eggs weighed nearly 10 g more at setting than RBC2 eggs, and the poults at hatching were approximately 8 g heavier than RBC2 poults (Table 1). The egg weight difference resulted in relative poult weights that were 1.5% greater in F than for RBC2 relative poult weights. The conductance constants for RBC2 were significantly greater than those of F suggesting that F embryos might retain more water during incubation but that difference in percentage water vapor loss was calculated to account for only 0.9% of the difference.

E Line vs RBC1 Embryo BW. E and RBC1 comparisons indicated the RBC1 hens laid a 15.1 g heavier egg than E; however, the poults weighed only 7.3 g more at hatching (Table 1). This difference resulted in a relative poult weight that was 2.9% greater in the E line than in the RBC1 line. The conductance constants differed between the two lines but the incubational water loss accounted for only 1.4% of the difference between the two lines.

Experiment 2

E Line hens had greater plasma thyroxine concentrations than RBC1 hens. Iodide supplementation did not increase plasma thyroxine or triiodothryonine concentrations in hens from the E and RBC1 lines, but it did increase the mean ratio of triiodothyronine to thyroxine. F Hens had lower thyroxine and greater triiodothyronine concentrations than RBC2 hens. Iodide supplementation increased thyroxine concentrations in both F and RBC1 hens but did not affect triiodothyronine levels, resulting in depressed ratios of triiodothyronine to thyroxine. These results confirm an effect of dietary treatment on the maternal thyroid.

F Line vs RBC2 Embryo Weights. Maternal iodide supplementation interacted with genetic lines to affect egg and poult weights (Table 2). Iodide increased egg and poult weights of the RBC2 line compared to those fed the basal diet but it had no effect on those of the F line. Despite significant differences in egg and poult weights, there were no differences in weight of the poult relative to the initial egg weights at setting. Embryo weight and growth rates for the F line embryos were greater than those of the RBC2 line beginning at Day 16 of incubation (Table 3) and throughout the remaining days of incubation. Maternal iodide supplementation decreased growth rates in F embryos beginning on Day 16. An interaction of line and iodide at Day 24 decreased RBC2 line BW compared to control but did not affect F line growth rates.

E Line vs RBC1 Embryo Weights. Maternal iodide supplementation increased egg and poult weights of the RBC1 line but embryo weights relative to egg weights were still greater in the E than RBC1 embryos. No line by iodide interaction was observed for relative poult weights. Iodide supplementation decreased growth rates of E and RBC1 embryos at Day 8 and increased them at Day 12 (Table 4). Line and iodide interacted at Days 16 and 20 to influence growth relative to hatchling mass. Maternal iodide supplementation increased growth rates in E embryos at Day 16 with no concomitant effect in RBC1 embryos: conversely, iodide supplementation increased growth rates of RBC1 embryos at Day 20 with no concomitant effects in E embryos.

Whole Body Glycogen Concentrations. The percentage of glycogen in whole embryos declined as the embryos grew (Table 5). Glycogen as a percentage of BW

TABLE 1. Egg and poult weights of turkeys from different genetic backgrounds

Line[1]	Egg weight	Poult weight	Conductance K	Poult weight/egg weight
	(g)			(%)
F	89.7[A]	59.8[A]	6.1[B]	66.9[A]
RBC2	79.9[B]	52.0[B]	6.6[A]	65.4[B]
Probability	0.0001	0.0001	0.003	0.0001
$\bar{x}$ ± SEM	84.8 ± 0.4	55.8 ± 0.4	6.3 ± 0.01	66.1 ± 0.3
n	(300)	(203)	(300)	(203)
E	66.4[B]	45.1[B]	5.9[B]	67.0[A]
RBC1	81.5[A]	52.4[A]	6.6[A]	64.1[B]
Probability	0.0001	0.0001	0.004	0.0006
$\bar{x}$ ± SEM	73.1 ± 0.3	48.8 ± 0.4	6.1 ± 0.01	65.2 ± 0.3
n	(300)	(227)	(300)	(227)

[A,B]Columnar means with no common superscript differ significantly ($P \leq 0.01$).

[1]F = turkeys selected for increased 16-wk BW; RBC2 = randomized population from which the F was derived; E = turkeys selected for increased 180-d egg production; RBC1 = randomized population from which the E was derived.

TABLE 2. Egg and poult weights of genetic lines of turkeys fed iodide

Line[1]	Diet[2]	Egg weight	Poult weight	Conductance K	Poult weight/ egg weight
		(g)			(%)
F	Iodide	87.4[a]	54.6[a]	6.3	64.6
	Control	86.0[a]	56.4[a]	6.1	64.7
RBC2	Iodide	80.9[b]	51.6[b]	6.4	64.9
	Control	74.7[c]	49.4[c]	6.6	64.5
	$\bar{x}$ ± SEM	82.0 ± 0.4	52.6 ± 0.4	6.4 ± 0.1	64.7 ± 0.3
	n	(320)	(181)	(320)	(181)
	Probabilities				
	Line	0.0001	0.0001	0.01	NS
	Iodide	0.001	NS	NS	NS
	Line × iodide	0.01	0.01	NS	NS
E	Iodide	62.1[c]	44.6[c]	5.7	66.7
	Control	63.5[c]	44.2[c]	5.9	67.3
RBC1	Iodide	79.8[a]	54.3[a]	6.0	65.6
	Control	78.1[b]	51.2[b]	6.4	64.2
	$\bar{x}$ ± SEM	72.2 ± 0.4	48.1 ± 0.4	6.0 ± 0.1	66.1 ± 0.3
	n	(320)	(182)	(320)	(182)
	Probabilities				
	Line	0.0001	0.0001	0.01	0.0008
	Iodide	NS	0.03	0.05	NS
	Line × iodide	0.05	0.01	NS	NS

[a,b]Columnar means with no common superscript differ significantly ($P \leq 0.05$).

[1]F = turkeys selected for increased 16-wk BW; RBC2 = randombred population from which F was derived; E = turkeys selected for increased 180-d egg production; RBC1 = randombred population from which E was derived.

[2]Iodide = basal maternal diet was supplemented with 4 ppm iodide; Control = basal diet.

was higher in the F than in RBC2 embryos at Days 8 and 12. Additionally, line interacted with iodide at Day 16 with RBC2 poults from the iodide treatment having less glycogen than the control, whereas there were no differences in the F embryos. The iodide supplementation reduced glycogen in both lines at Day 24.

Line E embryos had more glycogen at Day 12 than RBC1 and iodide supplementation decreased glycogen in both lines at Days 8, 12, and 24 (Table 6). Line and iodide interacted at Day 16 to decrease glycogen in E embryos with no similar effect on RBC1. At Day 20 the line by iodide interaction continued to elevate the glycogen in E embryos, but decreased it in RBC1 embryos compared to controls.

Embryo Livability. Hatchability of the F hens (66.0%) was significantly lower than that of the RBC2 (73.5%) but hatchability of the E eggs was significantly ($P < 0.0001$) better (80.0%) than that of the RBC1 eggs (57.9%). Dietary iodide supplemented to hens depressed the overall hatchability of all lines (71.8 vs 66.5%).

DISCUSSION

Embryonic Growth of Selected Lines

Embryos grew differently among lines of turkeys selected for 16-wk BW or 180-d egg production compared to the randombred control populations from which they

TABLE 3. Growth rates (percentage of hatchling mass) of turkey embryos (n = 24) from different strains when dams were fed supplemental iodide

		Day of incubation					
Line[1]	Diet[2]	8	12	16	20	24	28
F	Control	0.70	3.5	12.5	32.0	60.1	63.7
	Iodide	0.60	3.8	11.6	32.3	63.0[a]	63.9
	$\bar{x}$	0.65	3.6	12.0[b]	32.1[b]	61.6	63.8[a]
RBC2	Control	0.65	4.0	14.0	35.7	67.8[a]	52.0
	Iodide	0.67	4.0	12.4	36.9	60.7[b]	54.4
	$\bar{x}$	0.67	4.0	13.2[a]	36.3[a]	64.3	53.4[b]
	$\bar{x}$ ± SEM (n = 96)	0.62 ± 0.03	3.8 ± 0.01	12.6 ± 0.3	34.2 ± 0.7	62.9 ± 0.8	58.2 ± 1
	Line	NS	NS	0.03	0.006	0.001	0.0001
	Diet	NS	NS	0.03	NS	NS	NS
	Line × Diet	NS	NS	NS	NS	0.009	NS

[a,b]Columnar means with no common superscript differ significantly ($P \leq 0.05$).

[1]F = selected for increased 16-wk BW; RBC2 = randombred population from which F was selected.

[2]Iodide = basal maternal diet was supplemented with 4 ppm iodide; Control = basal diet.

TABLE 4. Growth rates (percentage of hatchling mass) of turkey embryos (n = 24) from different strains when dams were fed supplemental iodide

		Day of incubation					
Line[1]	Diet[2]	8	12	16	20	24	28
E	Control	0.87	4.6	13.2[b]	36.8[ab]	63.5	47.5[c]
	Iodide	0.59	5.4	15.8[a]	39.0[a]	70.0	43.1[d]
	$\bar{x}$	0.73[a]	5.0[a]	14.5	37.9	66.7[a]	45.3
RBC1	Control	0.68	4.0	12.2[bc]	33.0[b]	52.4	55.5[b]
	Iodide	0.57	4.4	11.1[c]	27.1[c]	57.7	59.9[a]
	$\bar{x}$	0.63[b]	4.2[b]	11.6	30.0	55.1[b]	57.7
	$\bar{x}$ ± SEM (n = 96)	0.65 ± 0.04	4.6 ± 0.14	13.1 ± 0.3	34.0 ± 0.8	60.9 ± 1.5	51.9 ± 0.8
	Line	0.04	0.01	0.0003	0.0001	0.001	0.0001
	Diet	0.03	0.04	NS	NS	0.07	NS
	Line × diet	NS	NS	0.009	0.01	NS	0.003

[a-d]Means with no common superscript differ significantly ($P \leq 0.05$).

[1]E = selected for increased 180-d egg production; RBC1 = randombred population from which E was selected.

[2]Iodide = basal maternal diet was supplemented with 4 ppm iodide; Control = basal diet.

were selected. The data from the current study suggest that selection for increased 16-wk BW increased egg and embryo weights compared to weights of the randombred control population from which it was derived. Beginning at Day 16 of incubation and continuing through hatching, F embryo growth exceeded that of RBC2. These differences existed mainly because of a 10 g difference in initial egg weight and a reduced conductance constant. In contrast, despite a 15-g difference in egg weights of E compared to RBC1 lines, no significant differences in embryo weights were noted until hatching. Differences in egg and poult weights and the relative weights at hatching between the E line and RBC1 suggest differences in embryo growth rates may be correlated with both egg weight and the metabolism as determined by eggshell conductance (Christensen and Nestor, 1994). These differences may exist to ensure that tissues mature properly during the plateau stage of incubation (Decuypere *et al*., 1992) and to ensure that the hatchling possesses the maturity to optimize offspring survival. If these suppositions are true, understanding them may improve our modern poultry management systems.

Embryonic growth in avian species may be determined by two primary factors, egg weight and length of the incubation period (Ricklefs and Starck, 1998). Ricklefs and Starck (1998) suggested that embryos from all species examined possessed amazingly similar developmental stages and differed only in degree of maturation at the end of the incubation period. The present study is the first to our knowledge that examined turkey embryonic growth.

Iodide and Lines. The second objective of the study was to determine the effect of iodide fed to the dam on the subsequent growth of the embryos. The maternal thyroid has been implicated in the growth of avian embryos of other species (Wilson and McNabb, 1997). Maternal iodide supplementation affected egg and embryo weights of each line differently. Iodide increased egg and poult weights in both randombred control lines but had no effect on the selected lines. Thus, it may be inferred that the different effects of iodide on the selected lines and their randombred controls occurs not only due to initial egg weight differences but due to embryonic growth as well.

The hypothesis was tested that genetic selection of turkeys for growth may interact with thyroid to result in

TABLE 5. Total body glycogen (percentage of hatchling mass) of turkey embryos (n = 24) from different strains when dams were fed supplemental iodide

		Day of incubation					
Line[1]	Diet[2]	8	12	16	20	24	28
F	Control	1,015	615	467[a]	147	94	11.7
	Iodide	1,009	567	455[a]	142	84	12.2
	$\bar{x}$	1,012[a]	591[a]	461	145	89	11.9[b]
RBC2	Control	706	575	451[a]	157	91	11.9
	Iodide	759	499	357[b]	111	74	14.8
	$\bar{x}$	732[b]	537[b]	405	139	82	13.5[a]
	$\bar{x}$ ± SEM (n = 96)	848 ± 31	564 ± 13	432 ± 9	139 ± 6	86 ± 4	12.8 ± 0.5
	Line	0.0002	0.05	0.007	NS	NS	0.05
	Diet	NS	0.02	0.01	0.05	NS	0.05
	Line × diet	NS	NS	0.04	NS	NS	NS

[a,b]Columnar means with no common superscript differ significantly ($P \leq 0.05$).

[1]F = selected for increased 16-wk BW; RBC2 = randombred population from which F was selected.

[2]Iodide = basal maternal diet was supplemented with 4 ppm iodide; Control = basal diet.

TABLE 6. Total body glycogen (percentage of hatchling mass) of turkey embryos (n = 24) from different strains when dams were fed supplemental iodide

		Day of incubation					
Line[1]	Diet[2]	8	12	16	20	24	28
E	Control	611	582	508[a]	156[a]	93	12.2[b]
	Iodide	410	488	359[b]	119[bc]	65	15.5[a]
	$\bar{x}$	511	506[a]	434	138	79	13.8
RBC1	Control	606	430	350[b]	116[c]	92	15.4[a]
	Iodide	439	414	386[b]	140[ab]	84	14.3[ab]
	$\bar{x}$	522	422[b]	368	128	88	14.8
	$\bar{x}$ ± SEM (n = 96)	469 ± 38	478 ± 14	401 ± 11	133 ± 5	83 ± 4	15.0 ± 0.5
	Line	NS	0.0008	0.009	NS	NS	NS
	Diet	0.05	0.04	0.02	NS	0.01	NS
	Line × diet	NS	NS	0.0006	0.006	NS	0.02

[a-c]Columnar means with no common superscript differ significantly ($P \leq 0.05$).

[1]E = selected for increased 180-d egg production; RBC1 = randombred population from which E was selected.

[2]Iodide = basal diet was supplemented with 4 ppm iodide; Control = basal diet.

different embryonic growth, as suggested for other species by many previous researchers (King and May, 1984; Stallard and McNabb, 1990; Hargis *et al*., 1991; Harvey *et al*., 1991). The present study may indicate mechanisms to enhance survival rates and simultaneously insure a hatchling of a size and a maturity that is characteristic of that line. The growth-selected (F) embryos actually grew at a slower rate than did RBC2 and egg production-selected (E) embryos grew at a faster rate than did RBC1. The data suggest that genetic selection of turkeys has affected embryonic growth rates independent of egg size.

The observations of numerous line by iodide interactions in the present study for growth, growth rates, or glycogen effects suggest strongly that the influence of maternal iodide supplmenetation on embryos is mediated by line. Depressed embryonic concentrations of thyroxine during pipping has been noted previously in turkey embryos with poor survival rates (Christensen and Biellier, 1982). Taken together, these observations may suggest that declines in hatchability as turkeys are selected for growth may be due to altered egg mass, eggshell conductance, or maternal and embryonic thyroids.

Whole Body Glycogen Concentrations

Line differences in glycogen metabolism were noted early in development for F/RBC2 comparisons and throughout development for the E/RBC1 comparison, possibly due to thyroid-mediated glycogen accrual and depletion (Nobukuni *et al*., 1989). Clearly, the interaction effects seen between line and iodide for growth rates suggests that increased growth rates of the E embryos was associated temporally with increased glycogen accrual or depletion, whereas in the F embryos it was not.

Summary and Conclusions

The data in the present study suggest a possible physiological mechanism for maternal and growth-related influences on embryonic survival. The data indicate that genetic line and maternal iodide supplementation can influence subsequent embryonic growth independent of egg weight, and may also affect embryonic survival. The data from the present study agree with previous studies in other avian species that have shown that thyroid hormones affect embryonic growth (King and May, 1984; Burke *et al*., 1990; Wilson and McNabb, 1997).

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